THESIS,

YELLOWS OF THE PEACH,

FOR THE DEGREE OF

B. S.,

IN THE SCHOOL OF NATURAL HISTORY,

BY

Albert Carver,

1889.

Introduction.

On entering the discussion of this subject, the writer will begin by saying that the information recorded in this thesis is not entirely original; but was obtained by a student under the guidance and supervision of his instructor. In pursuit of knowledge concerning the "Peach Yellows", I have found many species of bacteria, which probably take no part in producing the disease; but many of them are very curious, and interesting, so it seems fit to briefly mention and describe a few of them.

It is quite apparent to the writer that he has accomplished very little in his work of investigation although much time and labor has been involved. The growth of the organisms in the tissue is very slow, sometimes requiring several years for the trees to be entirely killed; and this subject has now been under consideration only about six months and that during the winter.

If the specific disease had been isolated and grown on artificial media, trees inoculated with it might not show the effect during the short period of investigation.

In as many cases as possible I have taken Photo-micrographs from the negatives taken by Mr. S. W. Stratton. These prints show the actual organisms which I succeeded in isolating, but they are in every case highly magnified.

The negatives were taken with a Zeiss's 1/2 in. apochromatic objective; compensating eye piece No 8;

and Carbutts ortho-chromatic plates: lamp light exposure 3 to 5 minutes. The organisms were in nearly every case stained with methyl violet. The magnifying power varied from 800 to 1000 diameters.

The Photo-micrographic prints, known as blue prints, are prepared as follows. A sensatizing fluid is prepared by making a solution of citrate iron and ammonium 1 oz. water 4 oz; another solution of red prussiate of potash 1 oz. water 6 oz. The sensatizing fluid is ready for use by adding equal quantities of these two solutions. They, however, should not be mixed until time for using the mixture. The solution is applied to the paper by means of a thin strip of board covered with cotton flannel which has considerable nap on its surface. It is applied evenly and smoothly, and in a few minutes it becomes dry. The paper must be kept in the dark, otherwise it will soon turn blue.

The Photo-micrographic prints are then made by placing a negative in the frame, over this a sheet of sensatized paper, then a heavy cover. It is then exposed to the direct rays of the sun 3 minutes. The paper after exposure is taken from the frame and washed. It is then hung up by one corner and left to dry. If successful we have a beautiful blue print of the microbes.

Peach Yellows.

The first symptoms of the disease are made apparent to the eye of almost any observer by the small yellow spots, or the pale yellow edges of the leaves. At first the spots are all pale, but finally assume a bright yellow color. The leaves also become more or less curled and enlarged. The twigs have a wiry appearance. The woody structure is more yellow, in contrast with that of healthy trees which is nearly white.

Young trees are susceptible to the disease, but more frequently it sets in after the tree begins to bear fruit. Generally some part of the tree, as a certain branch, first shows a sickly condition. If this branch is removed, the disease, the next year becomes apparent in some other branch, usually that nearest the removed portion. The bark on the body of the tree has no longer its bright green color, but assumes a yellowish decaying look, and in a few years the tree is entirely dead.

The writer observed the slow but sure work of a supposed disease in an orchard of peach trees near Springfield several years ago; but without the careful observations of an investigator. However many of the spectacle there presented are fresh in his memory.

Causes.

The cause of the peach tree disease has been attributed to many things, which by later investigators has been found to be erronious. Among the earliest statements as to the cause of peach yellows were those attributing the disease to climatic influences, such as frost, floods and drouths; but, they have been proven to be no more than modifying influences. Injuries by animals and insects are also among the disproved theories. Upon the same list is classed excessive cultivation, neglect of cultivation, and neglect of pruning. Also the "injuries to tap roots, propogation by buds rather than by seeds, defective drainage" &c. I think it can be quite conclusively stated that these agents may aid in the development of the yellows, but cannot cause the disease. To what then can it be attributed? The underlying cause is without doubt established as a living organism.

The spread of the yellows from one part of a tree to another; or from a diseased to a healthy tree seems to establish this fact. The great problem then is to find the specific organism causing the disease, isolate it, and establish the facts concerning it. This has been the object of the writer in his biological investigation; but how much he has accomplished in that line, if anything, time will tell, for Scientists will not long remain ignorant on this subject: although the work in itself is attended with many difficulties.

In the fall of 1888, Mr. E. F. Smith a special investigator of peach yellows at Washington sent eight diseased peach trees to Prof. T. J. Burrill of the University of Illinois for investigation. He had not yet made biological investigations of the diseased trees. These trees supposed to be effected with the yellows were sent from Dover, Delaware by request of the agricultural department at Washington. One of the trees was planted in a tub in the greenhouse that fall, and began to grow early the next Spring. The others were placed in the garden, the roots being covered with straw, and then earth.

The subject of this thesis is considered under three general divisions.

I. Appearance of the diseased trees.

II. Microscopical investigation of the tissues.

III. Biological studies upon the organisms found.

My work of investigating the yellows of said trees began January 17, '89. The first work was to cut thin sections with the microtome, of healthy trees, and of diseased tissues of the trees above named; note the difference in the sections by a microscopical examination, and if possible find the supposed parasite in the diseased tissue.

Twigs were cut from a healthy tree growing back of Prof. Burrill's residance. Small pieces from these twigs were imbeded in paraffin and thin sections made with the microtome.

In the same manner sections were made from the root and branches of the diseased tree in the greenhouse. The results of these examinations will be stated further on.

It is supposed there may be found in the diseased tissues injurious organisms which can not be found in the undiseased. If the disease is really caused by a parasite, it should be found, and by methods described further along introduced into the healthy tree to note the results. Proof is only attained when it is ascertained that the disease follows in consequence of this inoculation. The media used in the cultivation of the organisms found, were of two kinds. The preparation of each will here be briefly described.

I. Liquid media, or beef broth.
II. Solid media, or agar-agar, gelatin and Irish moss.

To prepare liquid media chop finely 1 lb. of lean beef, removing as much fat as possible, this is done to best advantage when cold, then soak over night in one liter of cold water. After soaking squeeze all the juices from the meat. Boil gently to coagulate the albuminous substances which may then be removed from the top of the liquid. Boil the residue lively for a few minutes until it turns white. Neutralize with $NaCO_3$, adding it drop by drop. Boil it gently a few minutes and filter, this leaves the liquid entirely clear, transparent and of a bright straw yellow color. Put the broth into small flasks and tubes closed with cotton plugs. The flasks and tubes of broth are then sterilized by putting them into a steamer at 90°C one hour daily for three or four successive days. When sterilization is complete the broth is ready for use.

The solid media, agar-agar, gelatin and Irish moss are prepared by adding to the liquid media a sufficient quantity (5 to 10 per cent) of Japanese sea weed glue or agar-agar, pure gelatin, or Irish moss respectively. When completely dissolved by boiling it is strained through a flannel cloth and sterilized in tubes and flask as before.

The cultures were grown in an incubator at almost a constant temperature, 38°C.

The organisms were in nearly every case stained with methyl violet. By so doing the microbes are more readily observed and can be more acurately described.

Fig. I. represents a highly magnified section of a twig from the healthy peach tree. The sections are stained with borax carmine, giving the tissue a beautiful red color, and making a fine structural display. The cell walls are regular and not broken or distorted. The protoplasm and starch granules are evenly distributed throughout the cells, and not even in the slightest degree coagulated. The contents of the cell are transparent as seen in the section.

In the cut, h, represents the cuticle; e, the epidermal cells; and p. parenchema.

Fig. II, represents a section from the root of a diseased peach tree. The section was given a beautiful stain with borax carmine. The cells are sometimes regular, and sometimes irregular and distorted as shown in Fig. III. They are filled with large granules of starch. Occasionally a cell is seen with only a few granules.

In other sections (Fig. II) many of the cells contain numerous exceedingly small granules which have the appearance of organisms. They stain bright-red — the tissue have a bright-yellow color. The presence of an organism in the tissues of the sections cut has not been confirmed.

Slide No. 94 is a mount from a culture in broth, inoculated with a bit of the tissues from a diseased peach tree. A small particles of the tissues was taken in sterilized forceps, and passed through the alcohol flame to sterilize the surface. It was then dropped into the tube of beef broth, and kept in an incubator at a temperature of 38° C.

On the fourth day a thick film or pelicle covered covered the surface of the liquid, which adhered to the walls of the tube. The surface of the pelicle was wavy and reticulated. Crest of the waves pure white. Liquid below the pellicle quite clear.

Fig. XIII. shows the bacilli after a growth of three days. Spores present but not shown in the Micrograph. Bacilli stained with methyl violet. They are seen in long and short rods. Length $2\frac{5}{7}\mu - 6\frac{5}{7}\mu$, width $\frac{10}{14}\mu$. Spores numerous, single, or in chains of twos or threes. All about the same size. $1\frac{1}{2}-\frac{5}{7}\mu$ (micros).

Slide No. 98. Mounted from a culture in broth, which was inoculated from the tissues of the diseased peach tree, as were all the following. Growth three days old, liquid cloudy with many flocculent particles suspended throughout the medium. Bacillus, as all the following, stained with methyl violet. Straight cylindrical rods, slightly rounded at the ends. Length varies considerable, but the average length is about $4\frac{2}{7}\mu$, width $\frac{10}{14}\mu$.

Spores numerous and in long chains. Edges and ends (the remains of the cell wall) deeply stained. Length $2\frac{6}{7}\mu$, width $1\frac{1}{4}\mu$. Fig. VI is a Micro-graph showing indistinctly the bacilli and spores.

Slide No. 106, from a growth on Irish moss. On the fourth day it covered about $\frac{1}{4}$th the surface of the medium. Proceeding irregular, amoeba-like. Surface reticulated. Bacillus not yet developed. Spores numerous, lying side by side or in chains. Remains of the old cell adhering to the ends of the spores and deeply stained. Length $1\frac{1}{3}-1\frac{1}{2}\mu$, width $\frac{1}{2}-\frac{3}{4}\mu$.

Slide No. 107, from a culture grown on Irish moss. Bacilli very small and numerous. Cylendrical, needle-shaped organisms. Deeply stained. Length $1\frac{3}{7}-2\frac{6}{7}\mu$, width $\frac{10}{14}\mu$.

Slide 109. Bacillus and spores present. Bacillus straight cylendrical rods, length occasionally four time the width. ~~Shown in Fig. XX.~~ Spores present. Length of those observed $\frac{3}{4}-1\mu$.

Slide No. 108, from a culture grown in broth. Pure growth of a Bacillus. Long needle shaped rods. Length $2\frac{6}{7}-5\frac{5}{7}\ \mu$, width $\frac{10}{21}\ \mu$. Shown in Fig. XX.

Slide No. 113, shows an organism found in a peach tree supposed to be healthy. Bacillus and spores present. Bacilli straight cylendrical rods. Some of the bacilli with truncate ends, others slightly rounded. Sometime connected in long chains, straight or serpentine. Bacilli various in length, some very long others short. Spores thicker than the rod. Length $2\frac{1}{7}\ \mu$, width $\frac{10}{14}\ \mu$.

Slide No. 114. A very small Bacillus. Short, and connected, sometimes in short chains. Ends rounded. Length $2\frac{1}{7}\ \mu$, width $\frac{10}{14}\ \mu$. Propagating by division. Deeply staine. Shown in Fig. IX.

Slide No. 119, examined on the 7th day. Liquid colored red. A long, fine thread-like mass filling filling a greater portion of the fluid. Pelicle formed on the surface but having a tendancy to sink.

A long straight cylindrical Bacillus with truncate ends. Organisms few in number.

Slide No. 120. The culture examined on the seventh day showed a thick pelicle, partially sunken beneath the surface of the liquid; but adhering to the walls of the tube. A long growth suspended from the bottom and center of the pelicles. Under the microscope there was found a very long and continuous growth, having very short joints, which after one or two days growth become broken up into individuals. The threads are about $\frac{10}{21}$ μ. in thickness. Stained deeply, seen in Fig. XV.

Slide No. 131. is a mount from a culture on solid medium which was inoculated from another growth on broth. Bacillus and spores present. Bacillus deeply stained. Straight cylindrical rods, ends slightly rounded. Length very various. Width $\frac{10}{14}$ – $\frac{10}{21}$ μ. Spores oval or oblong.

Slide No. 132 shows a very thread-like Bacillus, multiplying by division only. Straight cylindrical rods. Length various, but many times the diameter. Not deeply stained.

Slide No. 133. A very large bacillus. Propagating by division. Rods straight or curved with truncate ends. Sometimes connected in long chains. Length may be several times the width. Deeply stained. No spores observed. Width 1.9 μ. This organism is undoubtedly an "impurity" from the atmosphere and has nothing whatever to do with the disease of the peach tree. It is one of the most common microbes and is present almost everywhere — Bacillus figurans.

Slide No. 134, shown by the Micrograph (Fig. XV). The culture is a long continuous chain of a thread-like Bacillus. When young the joints are almost imperceptible, but with age the individuals become separated. The length varies from very short to those many times the thickness. Organisms deeply stained. No spores present. Thickness $\frac{10}{14}\mu$.

Slide No. 138 is a mount from tube No. 136, which was inoculated with a portion of the tissues of the bark of the diseased peach tree growing in the greenhouse. The tissues was taken from the branch about $\frac{1}{2}$ in. below the sawed limb, and was about $\frac{3}{4}$ in. long by $\frac{1}{2}$ in. wide. The surface of the tissue was sterilized by passing it twice or thrice through the alcohol flame. Then two incisions were made with a sterilized scalpel about $\frac{1}{4}$ in. apart, broken open, and some of the underlying tissues were taken out with sterilized forceps and introduced into beef broth. This process was supposed to prevent the introduction of any microbe except that in the tissues.

A microscopic examination of the liquid on the next day after inoculation, showed numerous short, cylendrical Bacilli. Mostly straight rods as shown in Fig. XIX, but sometimes slightly curved. Propagation by division. Ends truncate when division first takes place, but soon becoming rounded. Deeply stained. Length $1\frac{3}{7}$–$5\frac{5}{7}$ μ, width $\frac{10}{14}$–$1\frac{1}{4}$ μ.

Slide No. 149. Growth one day old. A short, straight, or curved Bacillus. Deeply stained. Length $1\frac{3}{7}$–$2\frac{6}{7}$ μ, width $\frac{10}{14}$ μ. Bacilli shown in the Micro-graph (Fig. VII.). Some of the mounts from the culture showed numerous spores. Small and eliptical. Length $1\frac{3}{7}$ μ, width $\frac{10}{14}$ μ.

Fig. X is a Micro-graph taken from slide No. 153. A very small micrococus, which seem to be in pairs.

Slide No. 202. A culture from a roll tube. The growth of the bacteria in the roll culture was very characteristic. It soon covered the whole surface of the medium, as a white film. Under the microscope is revealed a short

Bacillus, in some cases having an oval form, deeply stained and multiplying by division. No spore formation observed. Length $2\frac{1}{2}$–$5\frac{5}{7}$ μ. (Fig. VIII.

Slide No. 232. Impure culture, containing a large and small Bacillus, both deeply staine Large Bacillus, in length many time the thickness, shown in Fig. V. Width $1\frac{1}{4}$ μ. Small Bacillus oval, or oblong. Length $1\frac{3}{7}$ μ, width $1\frac{1}{4}$ μ, not seen in the Micro-graph.

Slide No. 241. A short oval Bacillus. Propagating by division. Organisms for a short time after division perfectly round, but becoming oval, or elongated later. Length $1\frac{3}{7}$ μ, width: Undoubtedly the same organism & shown in Fig. IX, but at a younger stage of development.

Slide No. 246. A microscopic examination showed a short cylendrical spore bearing Bacillus. Length varying. Ends decidedly rounded. Straight or curved. Length $1\frac{1}{7}$–$5\frac{1}{7}$ μ, width $1\frac{1}{7}$ μ.

Fig. XII is a Micro-graph taken from slide No. 172. It shows a pure culture of a Bacillus. Propagation by division. Ends truncate, and connected in long chains.

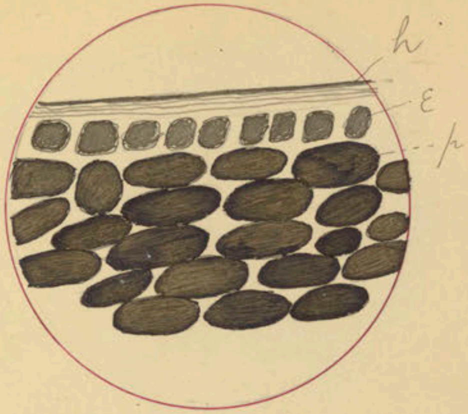
Fig. XVI is a Micro-graph from slide No. 183. This organism is by far the most important since it is supposed to have some close connection with the peach yellows. It was in three different cases isolated from the diseased peach tree and grown on artificial media. The organisms resemble very much those causing "pear blight" (Fig. XV), but the former are more elongated than the "blight." Four young healthy peach trees were inoculated March 25-89, with this organism, three of them by introducing the organisms beneath the bark — one by pouring water containing the microbes upon the earth about the tree. Up to the present time April 30, no sign of the disease is made apparent by the young trees. The young trees were planted in the greenhouse, and had begun to leave out when the inoculation was made.

Fig. XI shows, also a very small Bacteria which was isolated from the root of the diseased peach tree.

Fig. XVII and XVIII show two different kinds of micrococci which were isolated from the diseased peach tree, in the biological laboratory, but by another person.

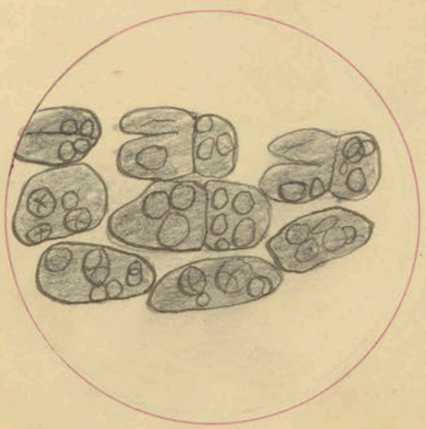
Many other slides were mounted, examined and discribed, but were found to be the same organisms which had been previously described and were therefore omitted.

I.



II.

III.



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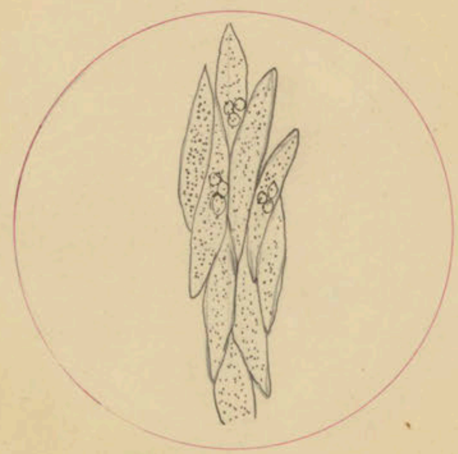

Fig V.

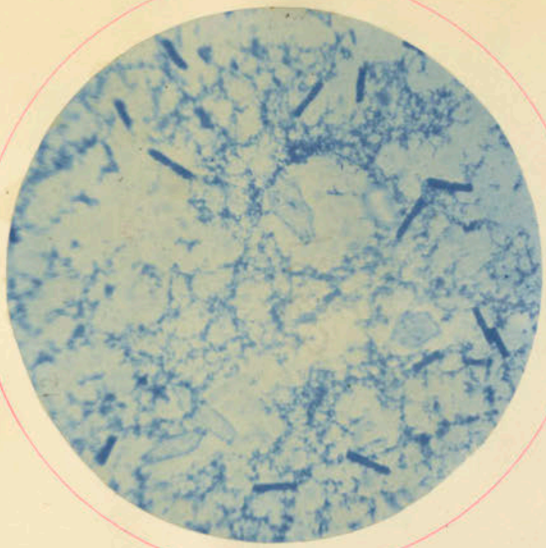

Fig VI.

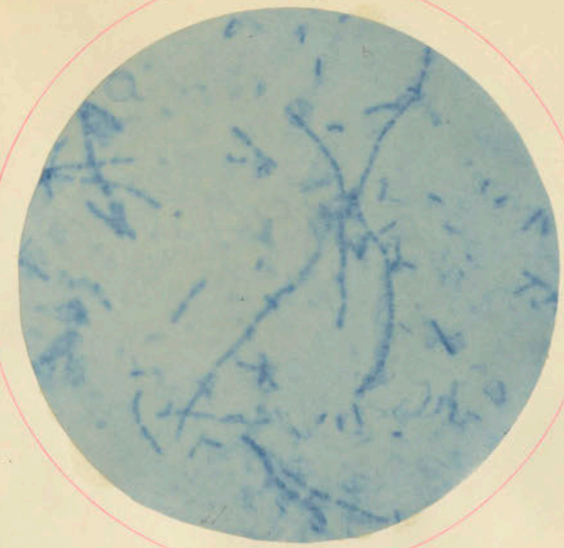

Fig. VII.

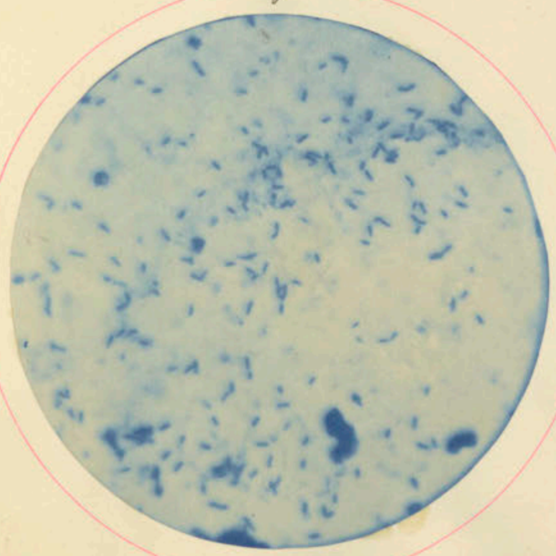

Fig VIII.

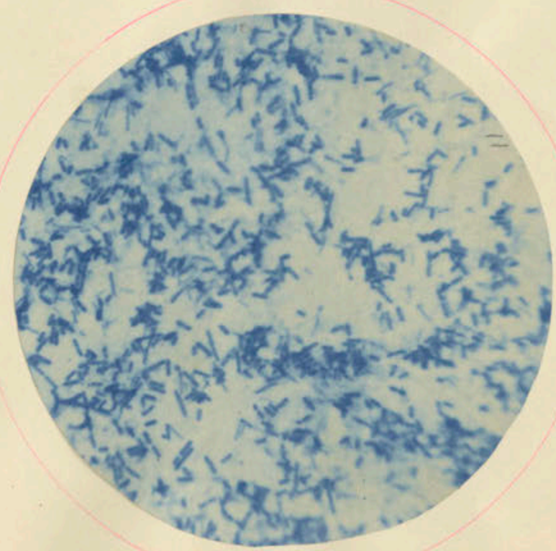

Fig. XIII.

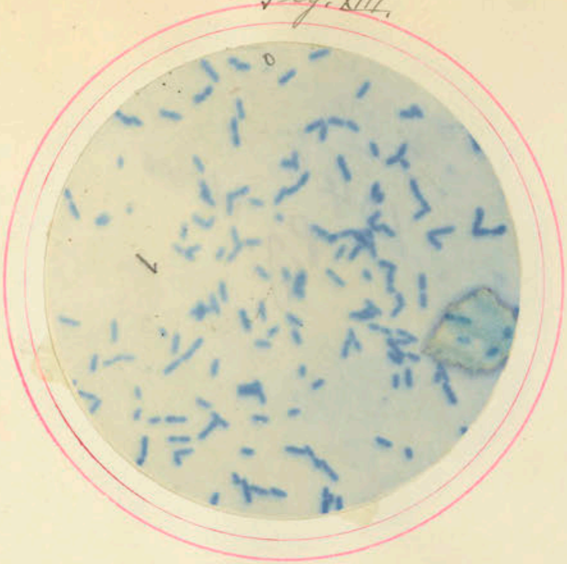

Fig. XIV.

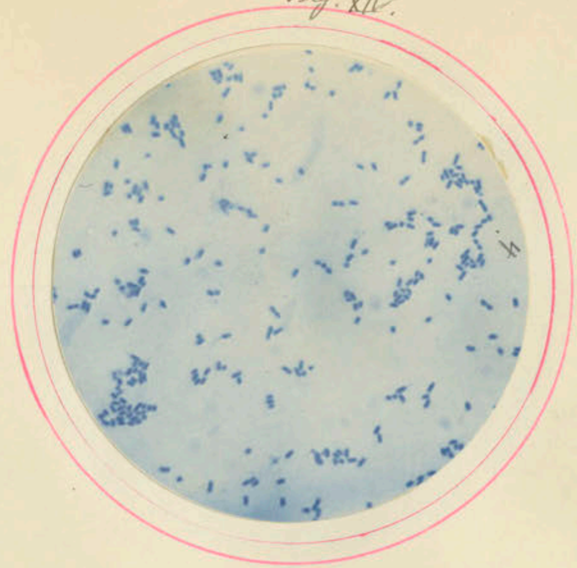

Fig. XV.

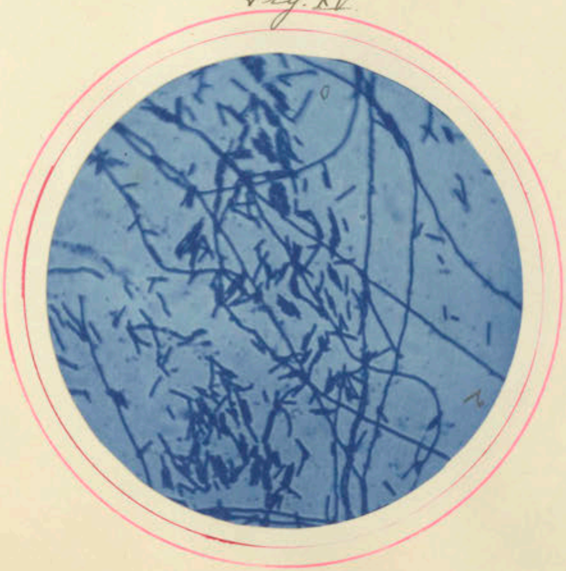

Fig. XVI.

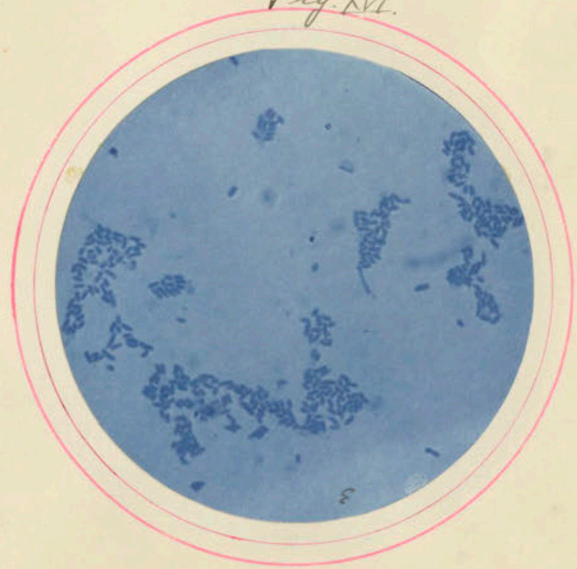

Fig XVII.

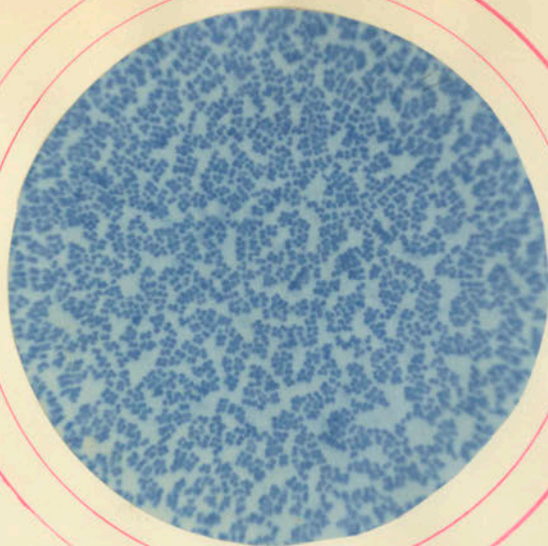

Fig. XVIII.

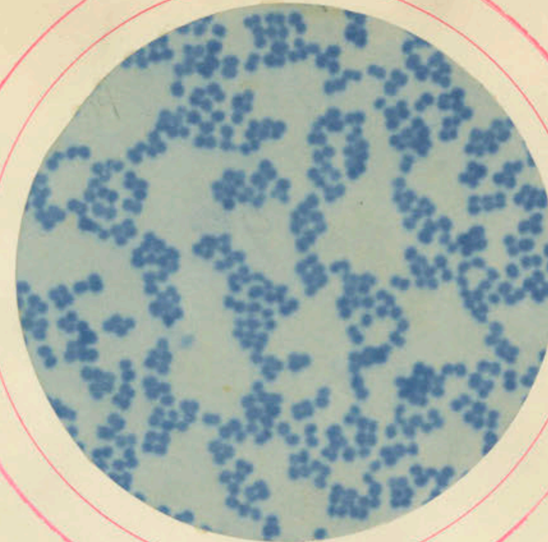

Fig. XIX.

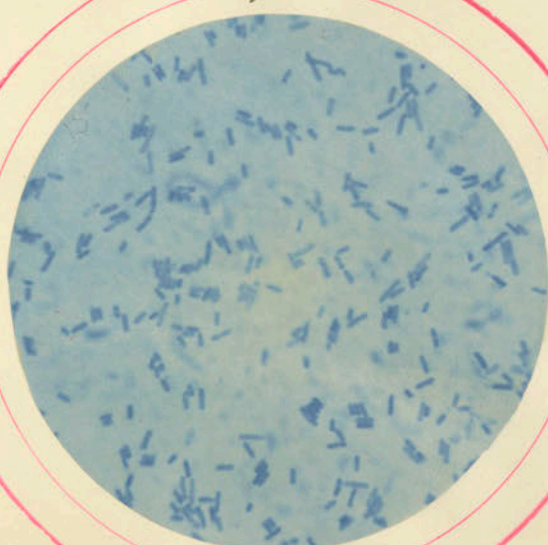

Fig. XX.

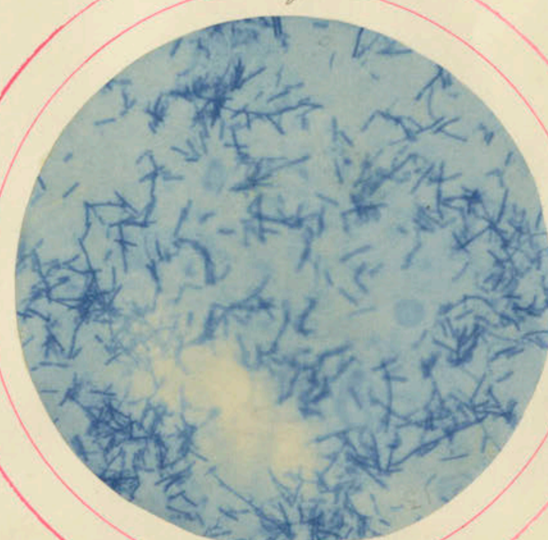

Fig. IX.

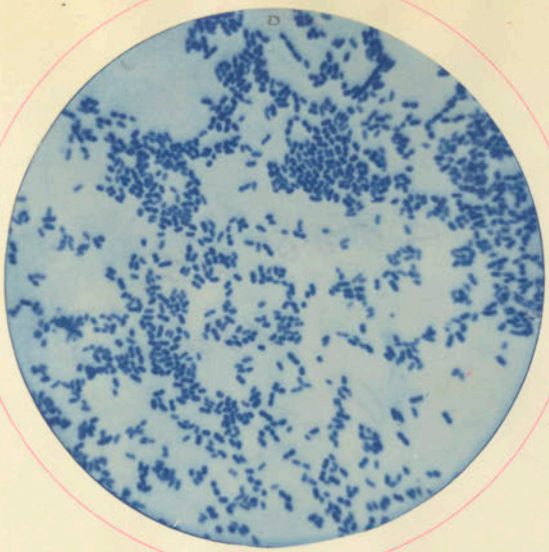

Fig. X.

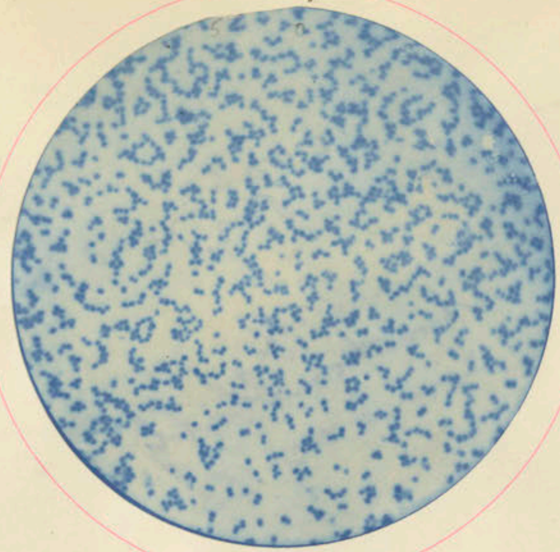

Fig. XI.

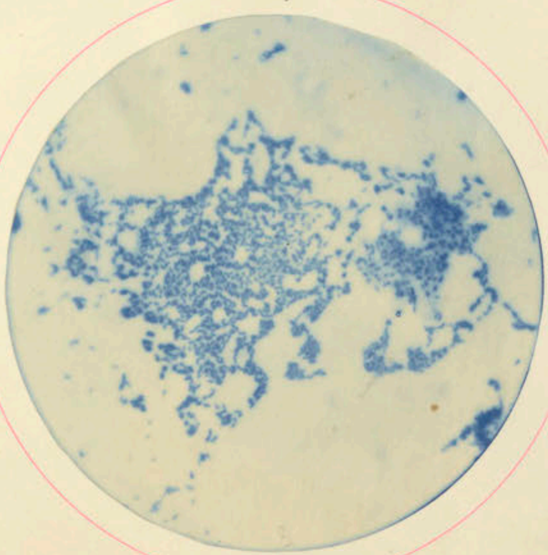

Fig. XII.